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Anti-RA33 Antibody is More Sensitive than Anti-Citrullinated Protein Antibody in Diagnosis of Rheumatoid Arthritis

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ABSTRACT

Background: To avoid Rheumatoid arthritis (RA)progression and disability, disease should be detected as early as possible, which needs diagnostic biomarkers which can dependably anticipate early RA and its progression, autoantibodies are one of the most reliable diagnostic markers that can be used. Anti-citrullinated protein/peptide antibodies (ACPA) are one of serological markers of RA. In addition anti-RA33 antibody (Ab) is another serological marker in RA but its diagnostic utility still dialectical, as it was present in a patients with small proportion.

Objectives: This research aimed to show the combination of anti-RA33Ab and ACPA diagnostic reliability in RA among Iraqi patients.

Methods: This is a retrospective case- controlled clinical study with a total of 360 subjects. ACPA and Anti-RA33 were measured by ELISA kits.

Results: The highest positive predictive values were occur in the ACPA with specificity of 98.8% while the highest negative predictive value were present in the Anti-RA33Ab with sensitivity of 76.1%.

Conclusion: The current results suggests that anti-RA33Ab has the highest diagnostic sensitivity value, so it could be regarded as important immunodiagnostic marker, but ACPA is more specific for disease.

Keywords: Rheumatoid arthritis, ACPA, Anti-RA33Ab.

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INTRODUCTION

Rheumatoid arthritis is a chronic autoimmune disease that essentially affects joints and cause swelling and pain. These symptoms usually worsen following rest. The most common joints involved are wrist and hands, with symmetrical pattern (1).

RA principally begins as a condition of continuous activation of immune cells promoting autoimmune response and immune complex formation at joints and affected organs level. The synovial membrane is the primary site of disease, where infiltration of immune cells followed the swelling and congestion of joints. During progression of RA three phases developed: initiation phase (non-specific inflammation), amplification phase (activation of T-cell), and chronic inflammatory phase, that result in destruction of tissue and cytokines production (IL-1, TNF-alpha and IL-6) (2).

The hallmark of autoimmunity is autoantibodies produced against self-auto-antigens. The cause behind antibody production still inadequately explained (3,4).

During the 1940s, Waaler developed the idea of autoimmunity in RA, who concentrate on the defects in metabolism of connective tissue occurred in this disease (5,6). Waaler exhibited that the autoantibody rheumatoid factor (RF) is raised in RA patients. These discoveries recommend activation of local antigen and immune response in the synovium of joints.

Later on more specific immunodiagnostic markers were needed with more specificity for RA lead to uncovering new markers ,for example anti-RA33, anti-keratin antibody(AKA), and later, anti-citrullinated antibodies, which demonstrated a satisfying degree of specificity in diagnosis of RA(6,7,8).

An investigation referenced that the occurrence of positive RF, AKA and ACPA were decreased in early RA however a comparative pattern was not seen for the counter anti-RA33 antibody (9).

The first anti-citrullinated antibodies in RA were found by Niehus and Mandema during 1964 (9). Previous studies also demonstrated that 48% of RA patients and just in 1% of healthy controls were positive for anti-citrullinated antibodies (10). Ongoing researches have additionally classified ACPA into ACPA1, ACPA2, and ACPA3 (11, 12). A 39–89% range of sensitivity and 50–99% range of specificity was revealed in RA diagnosis (12–16).

During 1989, new auto antibody was distinguished in RA patients, which was calledanti-RA33 antibody (17). Anti-RA33 present in about 15-35% of RA patients (19). The main restricting factor in utilizing Anti-RA33 in criteria for RA diagnosis is the extended range of sensitivity between 6% and 75% (20,21). The use of anti-RA33 autoantibody in RA diagnosis is a matter of doubt, because it was detected in a lowered percent of RA patients (17,22,23).

The current analysis explores the diagnostic validity of anti-RA33 in comparison to previous immunodiagnostic markers as ACPA, among Iraqi patients.

SUBJECTS AND METHODS

A Case-Control Study that included 360 subjects. The patients in the study attended Imam AL- Hussein medical City/ Rheumatology outpatient clinic at a period from November - 2017 to March -2018. The control group included 180 (23male and 157 female) their age range from

(26-70) years all of them underwent for clinical examination by rheumatologist to exclude any inflammatory disorder. The patients group were 180 (23male and 157 female) their age range from (26-70) years all of them diagnosed with RA according to ACR/EULAR 2010 by rheumatologist. Ethically ,the permission had been taken from Karbala health director, Imam AL-Hussein medical City/ Rheumatology Department and from the patients about collecting blood sample and using their data for research purposes (180 of them accepted whom enrolled in the study and 26 rejected), all of them had 6 criteria or more from 2010 ACR/EULAR and those Patients with RA disease had other rheumatological disease or other autoimmune diseases such as Psoriasis, ITP or OA , had recent surgery or wound or acute local inflammation had been excluded from the study. Blood sample about 5 ml was collected from patients and control and used for estimation of ACPA (IBL – Germany ACPA, AntiCCP-2-Ab ELISA kits) andAnti-RA33Ab (mybiosources -USA Anti RA33Ab ELISA kits). Data collected statistically analyzed by SPSS version 20.

RESULTS

The study included 360 subjects: 180patients (Female 157(87.22%) and Males 23 (12.78%), their age range (26 – 70 years) with mean ±SD (48.46 ± 10.65), the controls groups (180) female 157(87.22%) and male 23(12.78%), their age range (26 – 70 years). With mean ±SD was (48.84±10.58) as in table (1).

Table 1: The mean age and standard deviation of subjects.

Parameters \ Group	Patients N=180			Control N=180		
	NO	%	Mean±(S.D)	NO	%	Mean±(S.D)
Age in years	180	100	48.46 ± 10.65	180	100	48.84±10.58
Males age	23	12.78	51.65 ± 12.08	23	12.78	50.48± 8.73
Females age	157	87.22	47.99 ± 10.38	157	87.22	48.60±10.83

The mean and standard deviation of serum level for ACPA U/mland Anti RA33 Abng/ml were 109.76± 13.32 and 10.72±0.12 respectively for patients group versus 8.34±0.42 and 3.37±0.16 for control group with highly significant P-value as shown in table (2).

Table 2: The mean and standard deviation of serum level for ACPA $_{U/ml}$ and Anti RA33 Ab $_{ng/ml}$ to patients and control.

Parameters	Mean±(S.E) Patients No=180	Control No=180	sig
ACPA $_{U/ml}$	109.76± 13.32	8.34±0.42	<0.0001*
Anti RA33 Ab$_{ng/ml}$	10.72±0.12	3.37±0.16	<0.0001*

* significantp.value its < 0.05 .by using Mann-Whitney Test.

The AntiRA33-Ab sensitivity was 76.1%, specificity (96.1%), PPV(95.2), NPV(80) & accuracy (86.4) while AntiCCP-2-Ab the sensitivity that was 72.7% and specificity (98.8%), the PPV(98.4), NPV(78) & accuracy (85.8), the anti RA33-Ab for patients & Control group seem to have sensitivity, and accuracy higher than sensitivity and accuracy of ACPA with P-value< 0.0001 as shown in table (3).

Table 3: The sensitivity and specificity for ACPA and AntiRA33-Ab test.

parameter \ Group		Patients N	Patients %	Control N	Control %
Anti RA33-Ab	Positive	137	76.1	7	3.9
	Negative	43	23.9	173	96.1
Total		180	100	180	100
Sensitivity		76.1			
Specificity		96.1			
Positive predictive value		95.2			
Negative predictive value		80			
Accuracy		86.4			
ACPA	Positive	131	72.8	2	1.1
	Negative	49	27.2	178	98.9
Total		180	100	180	100
Sensitivity		72.7			
Specificity		98.8			
Positive predictive value		98.4			
Negative predictive value		78			
Accuracy		85.8			

2*2 table chi-square was used to detect PPV, NPV, accuracy, Sensitivity& Specificity.

DISCUSSION

The rheumatoid arthritis is a chronic inflammatory disease with indistinct etiological agents distinguished by symmetric peripheral polyarthritis. This disease often results in damage of the joint and physical disability (26). Our study reported the high statistical differences (P= 0.000) among ACPA serum level in patients of rheumatoid arthritis (109.76± 13.32) compared with control (8.34±0.42). Other authors reported 34(68.0%),(26±63.4) , 30 (66.7%), and (3.56±0.24) about ACPA antibodies serum level with a high statistical differences (<0.005) among patient of rheumatoid arthritis compared with control. (24,27,28, 35).

In addition, in present study there was a high significant (P= 0.000) association between Anti-RA33 antibodies (10.72±0.12) and rheumatoid arthritis susceptibility compared with control (3.37±0.16). Several studies reported by (24,28) whose found the Anti-RA33 about 29 (58.0%) and 25 (55.6%) respectively with a high significant (<0.005) associated with rheumatoid arthritis in patients compared with control.

In the current study, there was a significant statistical difference in the levels of anti-RA 33 and ACCP between the patients and control groups. Anti-RA 33 autoantibodies were positive in (137) patients (76.1%) with sensitivity (76.1%), in contrast other studies showed its sensitivity 7.3%, 40%-60%, and 58% (24,27, 29). However, the low sensitivity of these studies can be illustrated by the verity that present study population excluded early type of RA patients and focus about established RA. Another study conducted by (25,30) whose reported that anti-RA33 with sensitivity about (98%) and (93%), these results were higher than result of present study.

In addition, the specificity of anti-RA33 antibodies was (96.1%), other authors reported specificity of (69–96%) (7, 8, 16, 17, 22, 31). Also, other study conducted by Pena and Rondon (2016) who found that specificity of (100%) about anti-RA33. In contrast, few studies reported polemic data consist of 98% sensitivity and 20% specificity about anti-RA33 in patients group with rheumatoid arthritis (25). This is due to strong ethnical variations and depends on the disease duration as it is mostly positive in the early stages of the disease.

Also the current study found that anti-RA33 test can be utilized as a diagnostic evidence for rheumatoid arthritis estimation. In present study the positive and negative predictive value represented (95.2%) and (80), this result was higher than the study conducted by (7), who reported that negative and positive predictive value of anti-RA33 represented (32.7%) and (82.3%) respectively.

In the present study, the negative predictive value confirms the efficacy of the test in discrimination of RA disease from other similar diseases with small joints involvement. Furthermore, as it is stated by other authors, that antibodies of anti-RA33 are useful in the diagnosis of patients with RA who are negative for ACPA and rheumatoid factor (24).

In current study, the sensitivity and specificity values were (72.7%) and (98.8%) of ACPA test respectively. On contrary, from one study to another there is a differences in the sensitivity and specificity values of ACPA test. The study conducted by (32), the sensitivity and specificity were (53%) and (79%) respectively, while in (23), these values were revealed to be (71.9%) and (100%), respectively. Another studies found the sensitivity extent of 39–89% and a specificity of 50–99% for the diagnosis of RA (12, 13, 15). The explanation of low sensitivity in these studies can be due to the fact that the inhabitance of current study associated with established rheumatoid arthritis and exemption the early rheumatoid arthritis patients. Moreover, ACPA had the highest specificity than anti-RA33 and this is in correspondence with other studies reported by (33, 34).

Furthermore, in our study the values of positive predictive and negative predictive were (98.4%) and (78%) of ACPA respectively. This study was correspond with other study reported by (27) who found the positive predictive values and negative predictive values of (86.6%) and (71.1%) respectively. Another study reported by (29) who found the values of positive predictive and negative predictive were (0.78%) and (0.66%) of ACPA antibodies respectively, this result disagreement with result of present study.

CONCLUSION

Our study suggests that Anti RA33 antibodies have the highest sensitivity and ACCP antibodies have the highest specificity, so the Anti RA33 antibodies seems to be exemplify an extra marker for immuno diagnosis of rheumatoid arthritis.

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CONFLICT OF INTEREST

None

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